

Prevalence of Hpv Infection in the Lekoumou and Niari Departments (Congo Brazzaville)

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Prevalence; Human papillomavirus (HPV); Lekoumou; Niari Congo Brazzaville

1. Abstract

1.1. Objective: to determine the prevalence of HPV infection in women in Lekoumou and Niari departments.

1.2. Patients and Methods: We carried out a descriptive and cross-sectional study over a period of 7 months from January to July 2019 in the department of Lekoumou. 100 women ranging in age from 16 to 73 years old. The variables studied were as follows: age, marital status, level of education, risk factors for the onset of HPV infection, age at first sexual intercourse, number of sexual partners, parity, gesture. The multivariate analysis was done between age, number of level of instruction, parity, age of first sexual intercourse and number of sexual Partners. The statistical analysis and the data processing were carried out by the Excel 2016 software and the graph pad prism version 5 software. The statistical test used was the chi-square test.

1.3. Results: During this study we collected 100 women. The mean age of the patients was 34.6 ± 11.9 with ranges ranging from 16 to 73 years. The most represented age groups were that of 20-29 years (31%) and that of 30-39 years (29%). The most represented level of education was the college level in 53% of cases follow-up of the level of primary education in 25% of cases. Almost $\frac{3}{4}$ of our study population were married women (74%), single people represented only 22% of the study population. The mean age at first intercourse was 16.4 ± 2.3 with extremes ranging from 12 to 25 years. Almost all of the women (97%) had had their first sexual intercourse before the age of 20. The average number of

sexual partners was 5.3 ± 3.2 with extremes ranging from 1 to 15 partners, 53% of women had at least 5 sexual partners. The mean number of pregnancies was 4.9 ± 2.8 with extremes ranging from 0 to 13 pregnancies. Almost half of the women (49%) have carried between 4-7 pregnancies. The mean number of deliveries (parity) was 3.6 ± 2.4 with extremes ranging from 0 to 12 deliveries. 36% of our study population had between 4 and 7 deliveries. Bivariate analysis failed to find a statistically significant difference between age and number of sexual partners, between age of first intercourse and level of education and parity.

1.4. Conclusion: Cervical cancer is a public health problem. Prevalence of infection of HPV is relatively high in our context of low incomes countries. It is important to know this prevalence in order to assure prevention of cervical cancer.

2. Introduction

Human papillomavirus (HPV) is a virus belonging to a large heterogeneous family of small viruses called Papillomaviridae [1]. HPV infection is sexually transmitted (STI) and is ranked 3rd STI worldwide. To date more than 230 types of human and animal papillomavirus have been identified, 204 genotypes fully sequenced [2]. About fifteen HPV are oncogenic and responsible for cancerous pathology of the cervix [2]. The latter is a major public health problem. Indeed, cervical cancer is the 4th most common cancer in women in the world [3] and the leading cause of cancer death in women in Africa. Today, thanks to perfect epidemiological and molecular knowledge of HPV types and variants in developed

countries, the incidence of cervical cancer continues to decrease, unlike in developing countries, especially in Africa. sub-Saharan region where the incidence is increasing exponentially mainly because of the poor organization of screening and prevention policies [4]. In Congo, cervical cancer is the second most common cancer in women in terms of incidence and the second in terms of mortality [5]. Some epidemiological and molecular studies related to HPV and CCU have been carried out in certain departments of the country Ebatetou E.A. [6] and Boumba A. [7] showed the direct involvement of HPV 16, 18, 33 and 31 in the development of pre-cancerous lesions of the cervix, respectively. In the departments of Niari and Lékoumou, as well as all the other departments of the Congo, no studies have been carried out. It is in this context that we set ourselves the objective of determining the prevalence of HPV infection in the departments of Lékoumou and Niari.

3. Patients and Methods

We conducted a descriptive and cross-sectional study over a period of 7 months from January to July 2019 in the departments of Lekoumou and Niari. The analyzes were carried out in the Laboratory of Medical and Morphological Analysis of the General Hospital of Loandjili (HGL) and the Laboratory of Virology, Microbiology and Quality / Eco-toxicology and Biodiversity (LVMQ / ETB) of the Faculty of Sciences and Techniques du Maroc (FSTM). Our study involved a population of 100 women ranging in age from 16 to 73 years old. All of these women voluntarily benefited from a cervico-uterine sample. Sexually active patients aged 16 and over who have given informed consent for adults and parental consent for minors were included. We did not include patients who had undergone total hysterectomy as well as those who were menstruating. We carried out a simple random draw to constitute the size of our sample.

3.1. Sampling method

Each patient was placed in a gynecological position. After placing a sterile disposable speculum, two samples were taken:

- The first sample was taken using an Ayre spatula from the exo and endocervix. The collected cells were spread evenly and in a thin layer on a glass slide, then fixed with a cytological fixative spray (Spray name). Each blade was numbered.
- The 2nd sample was taken using a cytobrush from the exo and endocervix. The detachable head of the cytobrush was immersed in a vial containing a 10% buffered formaldehyde solution. Each bottle was numbered.

3.2. Storage and transport of samples

After collection at the Sibiti base hospital, Komono and Mayéyé CSIs, the samples were transported to the Pointe-Noire LGH. Storage was at -20°C .

The transport to Morocco was done in dry ice by plane, in the hold, according to the WHO recommendations for the transport

of biological samples. In Morocco, the samples were immediately returned to -20°C in order to maintain the cold chain.

3.3. Molecular analysis

The molecular analysis took place in 4 steps:

- DNA extraction
- The evaluation of the DNA extract
- The detection of HPV viral DNA
- HPV genotyping

3.4. DNA extraction

The DNA extraction took place in 5 phases

- First phase: sample pretreatment

The cells were collected in a 1.5 mL eppendorf tube, to which were added 250 μL of phosphate buffered saline (PBS). We carried out two successive washes with PBS, by centrifugation at 12,000 rpm for 15 min at 4°C .

- Second phase: enzymatic digestion
- Third phase: purification with phenol chloroform
- Fourth phase: precipitation
- Fifth phase: washing and rehydration

3.5. Evaluation of the DNA extract

4-2-1-Qualitative evaluation of the DNA extract by β -globin PCR
All PCR amplifications were done in a GeneAmp® PCR thermal cycler

3.6. Detection of HPV viral DNA

4-3-1-Revelation

3.7. HPV genotyping by specific PCR

3-4-1-Analysis of specific PCR products

The variables studied were as follows:

- age,
- marital status,
- the level of education,
- the age of first sexual intercourse,
- the number of sexual partners,
- parity,
- the frequency of HPV infection

Bivariate analysis was done between age, education level, age at first intercourse, number of sexual partners, marital status and parity.

4. Statistical Analysis

All the results of this study were statistically analyzed from the Chi-square test or Fisher's exact test using Epi-Info™ software version 7.1.1.14, USA (<http://www.epiinfo.com>). For all tests, the association between two variables was considered statistically sig-

nificant when $p < 0.05$.

5. Results

During this study we collected 100 women. The mean age of the patients was 34.6 ± 11.9 years with ranges ranging from 16 to 73 years. The most represented age groups were that of 20-29 years (31%) and that of 30-39 years (29%) (Table 1). The most represented level of education was the college level in 53% of cases (Figure 1). Almost $\frac{3}{4}$ of our study population were married women (74%). Singles represented only 22% of the study population (Figure 2). The mean age at first intercourse was 16.4 ± 2.3 with extremes ranging from 12 to 25 years. Almost all of the women (97%) constituting our study population had had their first sexual intercourse before age 20 (Figure 3). The average number of sexual partners was 5.3 ± 3.2 with extremes ranging from 1 to 15 partners, 53% of the women had at least 5 sexual partners (Table 2). The mean number of deliveries was 3.6 ± 2.4 with extremes

ranging from 0 to 12 deliveries. 36% of our study population had between 4 and 7 deliveries (Figure 4). Out of 100 cases of women studied, HPV viral DNA was identified in 29 cases, for an overall prevalence of infection of 29%. (Table 3). HPV infection was observed in 58.3% in women aged 50 and over while only 12.5% of cases in women under 20 were infected (Figure 5). No difference significant was observed. $p = 0.15$; $\chi^2 = 2.073$ between the level of instruction and the infection with HPV (Figure 6). Although 50% of the widows of our population are infected by HPV, we did not observe a significant difference between the carrying of the infection and marital status. $P = 0.91$; $\chi^2 = 0.013$. (Figure 7). There was no association between age at first intercourse and HPV infection (Figure 8). No significant difference was observed ($p = 0.47$). $\chi^2 = 0.522$ between the number of sexual partners and HPV infection in (Figure 9). There was no association between the distribution of HPV infection by number of deliveries. The difference was not significant with a $p = 0.16$. $\chi^2 = 1.985$ (Figure 10).

Table 1 : Distribution of patients according to Age

Age Group	Number	Percentage
<20	8	8%
20-29	31	31%
30-39	29	29%
40-49	20	20%
≥ 50	12	12%
Total	100	100%

1- Sociodemographic characteristics ; Age

Table 2 : Distribution of patient according to sexual partners

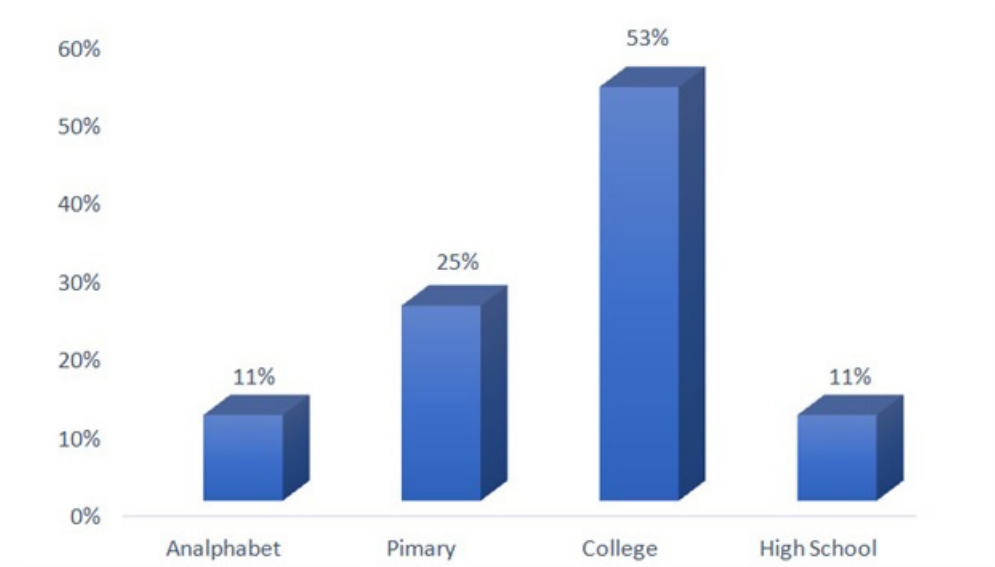
Partners	Number	Percentage
1 to 2	19	19%
2 to 4	28	28%
\geq to 5	53	53%
Total	100	100%

Sexual partners

Table 3 : Distribution of patients according to prevalence of infection of HPV.

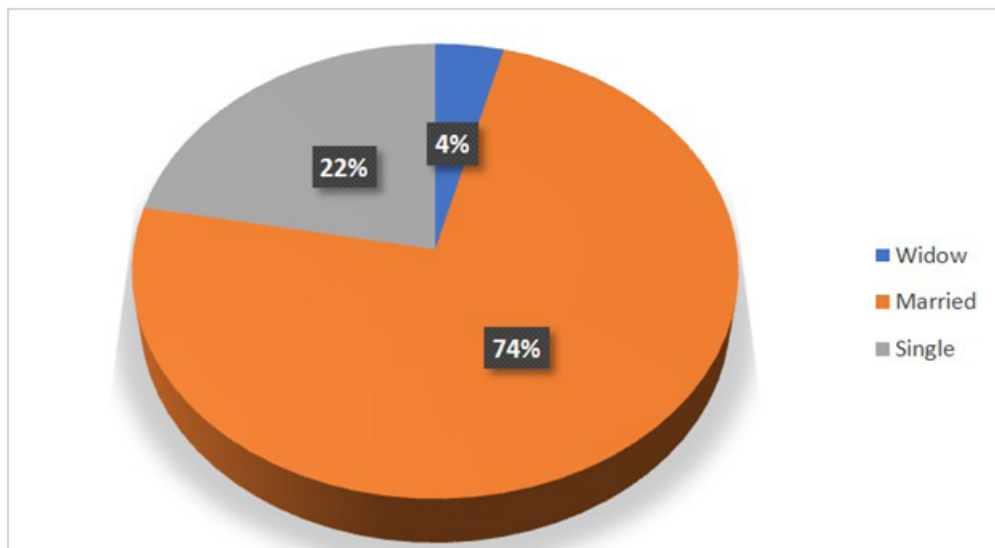
HPV	Number	Percentage
Negative	71	71
Positive	29	29
Total	100	100

2-Prevalence of infection of HPV



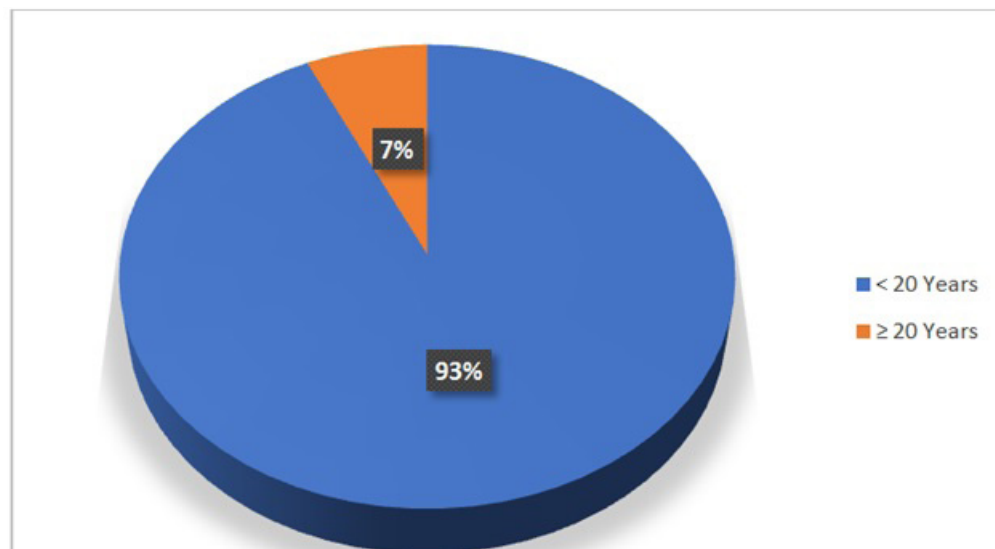
Level of instruction

Figure 1: Distribution of patients according to level of instruction



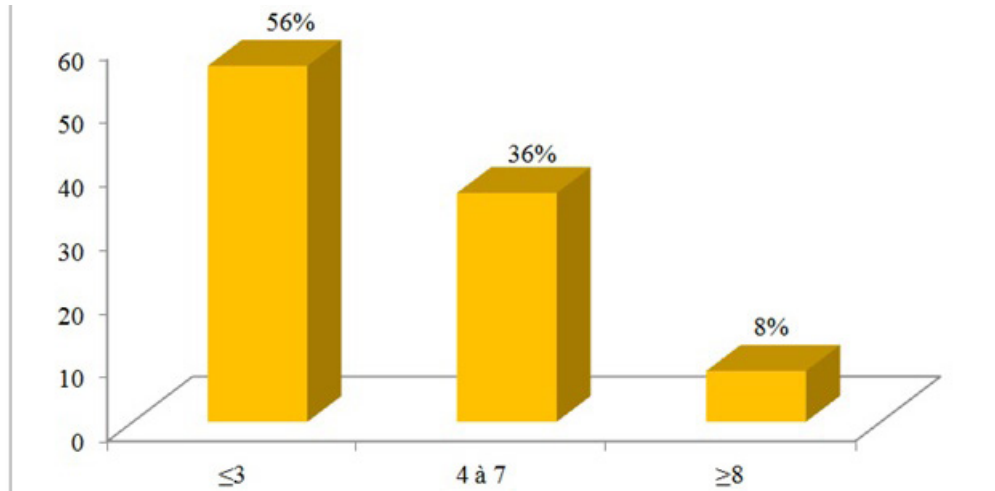
Marital Status

Figure 2: Distribution of patient according to marital status



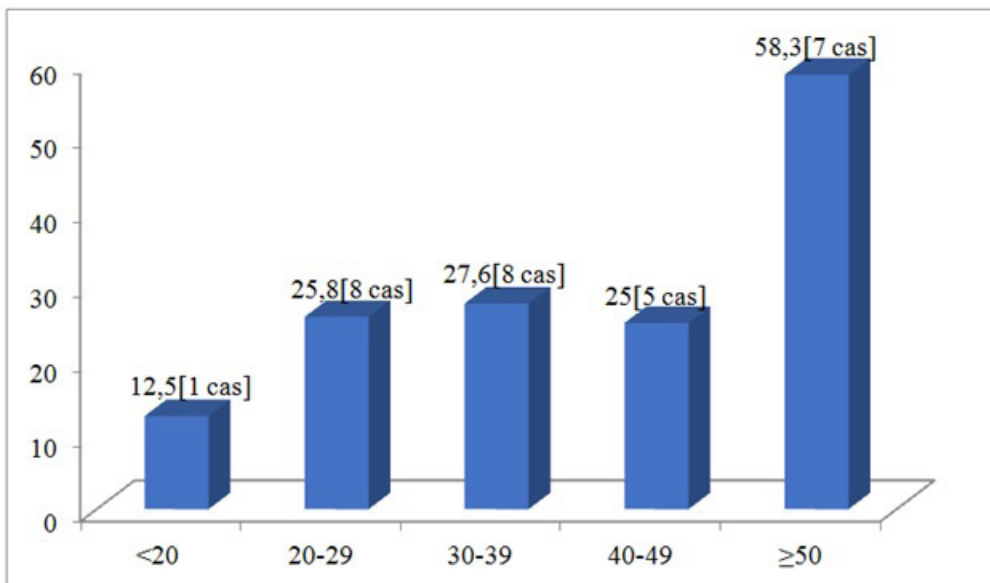
Age of first intercourse

Figure 3: Distribution of patient according to age of first intercourse



parity

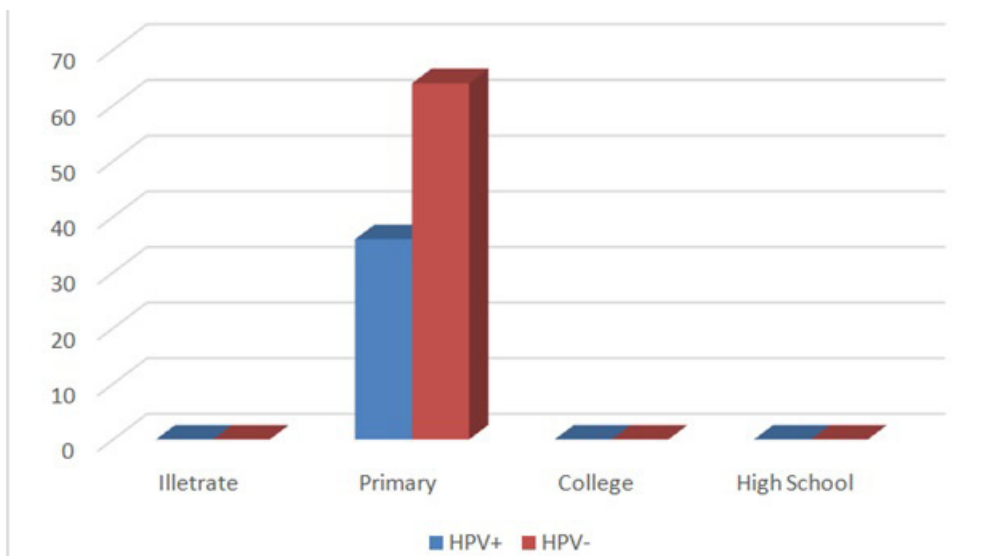
Figure 4: Distribution of patient according to parity



3-Risk factors of infection of HPV

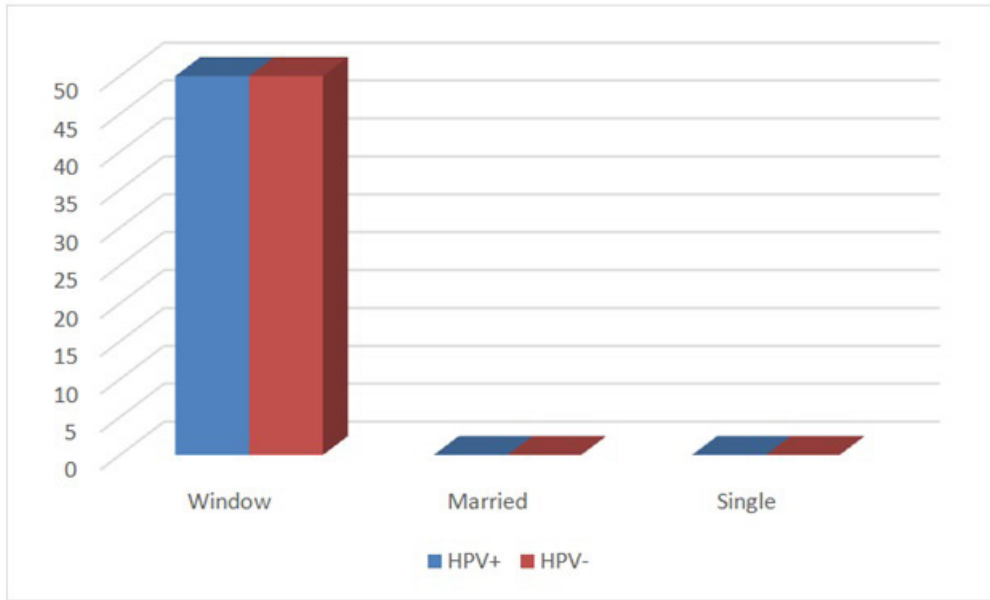
3-1-Age

Figure 5: Distribution of patients according to infection of HPV and Age



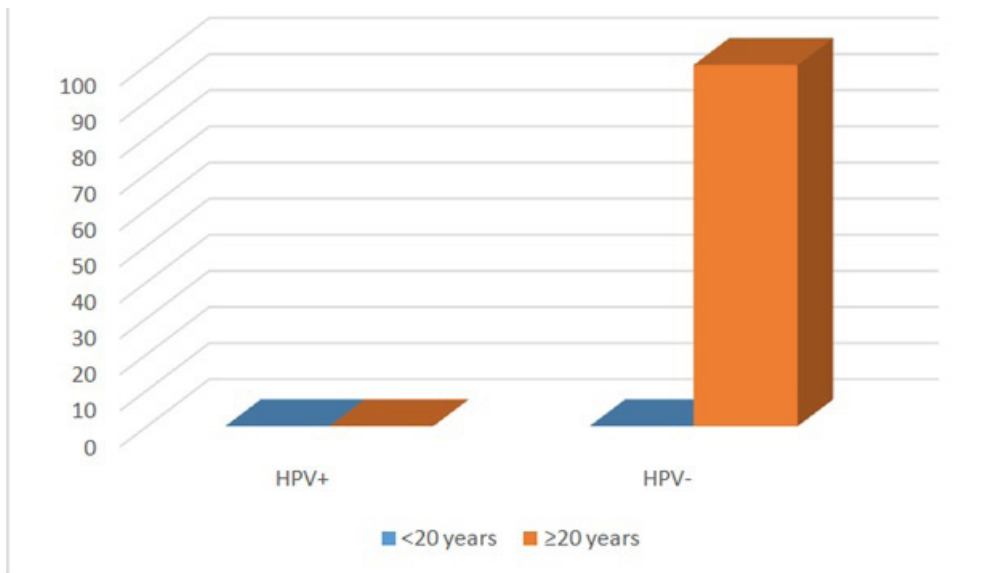
3-2-Level of instruction

Figure 6: Distribution of patients according to infection of HPV and level of instruction



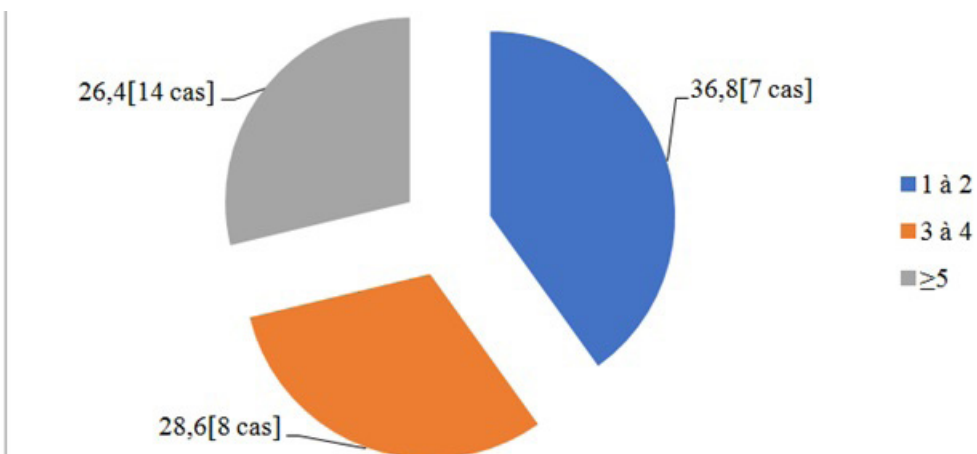
3-3-Marital status

Figure 7: Distribution of patients according to infection of HPV and marital status



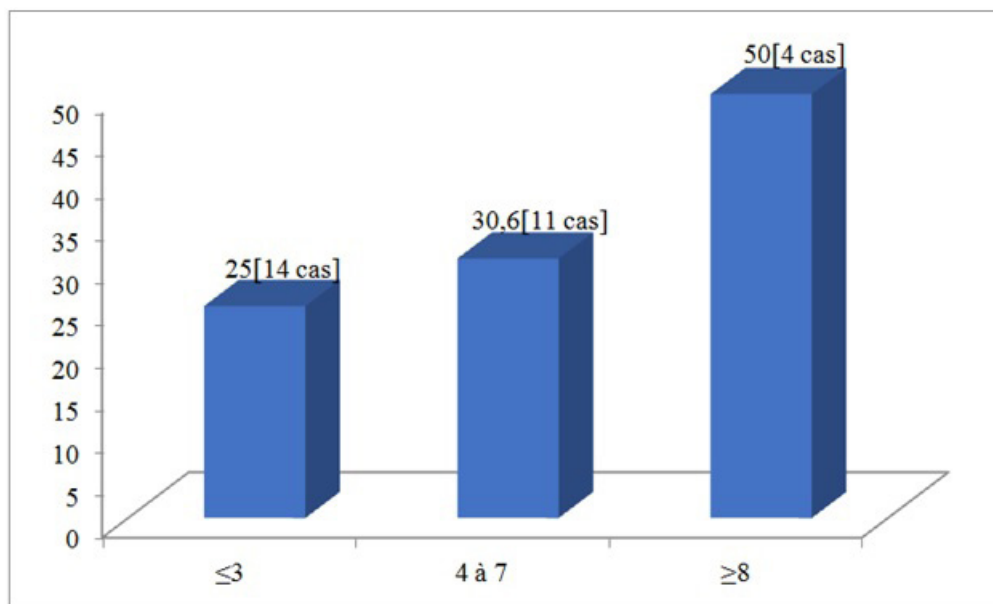
3-4-Age of first intercourse

Figure 8: Distribution of patients according to infection of HPV and Age of first intercourse



3-5-Number of sexual partners

Figure 9 : Distribution of patients according to infection of HPV and number of sexual partners



3-6-Parity

Figure 10 : Distribution of patients according to infection of HPV and parity

6. Discussion

6.1. Analysis of the methodology

6.2. Type and period of the study

The descriptive and transversal nature of this study ensures an optimal quality of the results, because the collection of information was contemporaneous with the events described. The homogeneity of our study sample allowed us to make a simple analysis of the results obtained, representative of the general population of the study area.

6.3. Study population

The selection criteria were established in order to avoid bias in the interpretation of the results. We performed cervico-uterine smears (CDU) in women aged 16 years or older. For adolescents aged 16 to 18, informed consent from a parent or guardian was required to be part of the study, while those over 18 had to consent themselves. Quite similar criteria were also used by Xavier Castellsagué in 2006 [8], in a study on the detection of the HPV genotype in rural areas in Mozambique. The mean age of women in our study population was lower than that observed in the Louie study population in 2009 [9] which was estimated to be 33.9 years.

6.4. Sampling strategy

The constitution of the sample consisted in making a simple random selection of women from the entire rural area of the Kouilou department, representing our sampling territory. The purpose of this random selection was to obtain a number of cases representative of the general population, in order to ensure statistical significance of the results. In total, we selected 100 cases for our cytological and molecular studies of HPVs. Elsewhere, but close to us in Kinshasa, in the Democratic Republic of Congo (DRC), Ali-Risasi in 2008 [10] using the same methodology, had recruited 272 cases

to carry out this type of molecular study of HPV identification in the area. urban.

6.5. Sampling method

Like the other teams, including Ali-Risasi in 2008 [10], we used liquid phase cervico-uterine smear samples. It consisted of rotating the cytobrush 3 times for each sample and this allowed us to have a homogeneous study sample, collected under the same conditions. The density of the cells obtained was perfect in all the samples, between 1.8 and 2. Currently, the liquid phase smear with the marketing of transport and preservation media has improved the conventional smear. The advantage of the liquid phase smear with cytobrush sample is that it allows both cytomorphological and molecular study to be done without having to use a second sample.

6.6. Choice of study methodology

According to Mougin in 2001 [11], epidemiological studies carried out around the world have shown that HPV research should take place well upstream of screening for dysplastic lesions and the revelation of cervical cancer. Indeed, the detection of HPV infections makes it possible to better identify populations at risk, with the aim of carrying out better surveillance, and therefore good prevention against this scourge. Currently, in the absence of a serological test, molecular biology techniques remain the only reliable tools for detecting viral DNA in a biological sample.

The genotyping technique was motivated by the objective and purpose of our study: that of studying the genomic variability of HPV 16 strains in our study population. Despite many existing genotyping techniques, only sequencing can achieve this goal by studying mutations directly on a fragment of the sequenced gene. Indeed, sequencing remains the genotyping technique par excellence today and is used in several studies around the world,

6.7. Prevalence of HPV infection

In a sample of 100 women, our study found a prevalence of HPV infection of 29%, of which 18% were HPV type 16 (HPV-HR).

In Africa, the work carried out on the genotyping of HPV strains has found very high frequencies; indeed, Castellsagué et al [8] in Mozambique found a prevalence of 36% in 2001 with respective frequencies for subtypes 16, 18 and 45 of 15%, 13% and 8%, Didelot-Rousseau et al [12] in Burkina-Faso found frequencies of HPV infection of 62.7% including 36.4% of high-risk subtypes (7.8% for HPV 16 and 6.4% for HPV 18) and a meta-analysis published by Clifford et al [13] noted a frequency of HPV infections in sub-Saharan Africa of 25.6% including 68.6% of HR HPV (8% for HPV 16, 4 % for HPV 18 and 6% for HPV 45) in women with normal cytology.

In our study, the HPV-HR consisted exclusively of type 16. This rate remains within the limit of most studies carried out in Africa on the general population which is 54.6% according to Munoz et al in 2003 [14] and 52 , 9% according to Didelot-Rousseau et al in 2006 [12]. In Congo, the only molecular studies on HPV have been carried out by Boumba [7], he focused on the genotyping of oncogenic HPV in circulation in Congo-Brazzaville, with the result of a prevalence of HPV 16 of 62.12% in an urban population of the city of Pointe-Noire. We note that this prevalence is close to that which we observed, thus confirming the high prevalence of HPV 16, a high risk oncogenic virus in the rural population of Kouilou. We were thus able to conclude that the HPV 16 virus in these two areas (urban in the study by Boumba , rural in ours) is present at high rates.

6.8. Analysis of HPV infection and associated risk factors

Several studies have shown that certain factors such as sexual behavior, parity, pregnancy, age, marital status, level of education are the risk factors most involved in the development of precancerous and cancerous lesions of the cervix. uterus. In our study, the statistical analysis between the majority of these risk factors (in particular parity, age, marital status and level of education) and HPV infection (viral DNA positivity) n 'showed no significant difference. This result, although contrary to most studies around the world, does not call into question the association between these risk factors and the carriage of HPV infection. Indeed, these risk factors are reported in the literature as risk factors associated with HPV infection and these complications [15]. The non-significance of certain risk factors in our study could be explained on the one hand by the fact that certain risk factors require a very large sample to be significant and on the other hand by the fact that some of these factors are markers of sexual activity more than cervical HPV lesions according to Mougin in 2001 [16] and Woodnam in 2001 [17]. Only the age of the first sexual intercourse (less than 20 years) is statistically significant for all the risk factors studied, these observations have been noted in several studies in the world,

those of Castellsague [8] and Munoz [14] for example.

7. Conclusion

Cervical cancer is a public health problem apart from HPV infection which is the risk factor there are some co-factors such as parity, age at first intercourse. The prevalence of HPV infection is relatively high in the Lékoumou and Niari regions. This high prevalence may justify primary and secondary prevention against cervical cancer in our resource-constrained setting.

8. Conflict of Interest

Where were no conflict of interest during this study.

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